Studies on Characterization and Bioactivities of Biosurfactant from Halophilic Bacteria

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Abstract:

The problem of emerging of resistant pathogenic bacterial strains to emerging antibiotics and demanding of a nontoxic antifungal compounds leading to search and developing of new classes of antimicrobial, anti-biofilm and antifungal compounds. Biosurfactants are surface active compounds that display a range of physiological function which have activity as antimicrobial and anti-biofilm. In this study, we proposed to characterize the biosurfactant produced by isolated halophilic bacteria and examine its role in inhibition of growth pathogenic bacteria, fungi and the inhibition or disruption of biofilm forming potential of microbial pathogens.

Introduction:

Surfactants are substances that absorb to and change the conditions prevailing at interfaces. These amphiphilic compounds partition in the interface between two phases differentiate in degrees of polarity and hydrogen bonding, such as the air and water or oil and water interface (Seydlová et al., 2008). Leading to reducing the surface and interfacial tension in aqueous solution and hydrocarbon mixtures (Jahan et al., 2019; Donio et al., 2013a). Surfactant, which is produced by microorganisms, are usually called biosurfactant (Banat et al., 2010). Biosurfactants have physicochemical properties beside its biological activities and environmentally accepted, low

toxic, biodegradable and effective in various extreme conditions (Liu et al., 2019; Jain et al., 2012). Additionally, biosurfactants can have low critical micellization concentration (CMC) values comparing with synthetic surfactants, which is improving their efficiency in various applications (Jahan et al., 2019).

Depend on the origin and chemical structure the biosurfactants are classified as lipoproteins, glycolipids, lipopeptides, phospholipids, fatty acids and polymeric molecules (Yaraguppi et al., 2020). Furthermore, they are generally classified as low molecular weight (involving lipopeptides and glycolipids) and high molecular weight (polysaccharides, proteins, lipoproteins, among others) biosurfactants (Gudina et al., 2013). Microbial genera reported as a biosurfactant producers include Arthrobacter, Acinetobacter, Pseudomonas, Bacillus, Halomonas, Enterobacter, Rhodococcus, yeast species, etc. Among them, Bacillus species are commonly found as a producer of lipopeptides (Yaraguppi et al., 2020). While biosurfactants used as emulsifiers, moistening, dispersing, and foaming agents. In addition, the biosurfactant are the best environmental compatibility at extreme temperatures, salinity, pressure and PH (Barakat et al., 2017). For that, extremophilic microbial surfactants appear to depend on the use of plenty and cheap substrates for optimization the process of the cultivation conditions, since they can adapt and maintain stability in adverse environments (Makkar et al., 2011; Barakat et al., 2017). Therefore, microbes from extreme environments have attracted attention in recent years to produce biosurfactants .This is due to the secret that they carry the molecular evolution of life and stability of the macromolecules (Donio et al., 2013b). The potency of marine microorganisms' in differential metabolic and physiological capabilities have been described as a determinant factor for their potential in secondary metabolites of industrial interest, including biosurfactants, and in the last years many studies were conducted for prospection of biosurfactant producers using

samples from marine environments (Pessôa et al., 2019). Halophiles may have an important role to play as surface-active agents due its lipid composition such as the archae bacterial ether-linked phytanyl membrane lipid of the extremely halophilic bacteria, which has been shown to have surfactant properties (Sarafin et al., 2014). Biosurfactants have a wide range of biotechnological applications in different areas such as cosmetics, food, dairy, beverage, detergent, textile, petroleum recovery, paper pulp, paint, mining, and pharmaceutical industries (Jain et al., 2013). In pharmacological field, the biosurfactants used as antibacterial, antifungal, antiviral, anticancer, immunomodulator, anti-adhesive against pathogens, probiotic nature, antioxidants, stimulate dermal fibroblasts, vaccines and gene therapy (Donio et al.,2013b; Gudina et al.,2013). In the last two decades, searching for the possibility to develop new classes of antimicrobial compounds has emerged due to pathogens acquired resistance to emerging antibiotics (Sharma et al., 2014). There are biosurfactant groups are known to have antimicrobial activity, including lipopeptides, glycolipids & lipoproteins (Arifiyanto et al., 2020). Furthermore, it can be used to control biofilms which have shown to alter the surface properties of bacterial cells and reduce their adhesive properties and biofilm formation (Sambanthamoorthy et al., 2014; Giri et al., 2019).

Literature reviews

Jain et al., (2012) has studied on production of biosurfactant from an alkaliphilic bacterium *Cronobacter sakazakii* (accession no. JN398668) which is isolated from total of 43 isolates were collected from Alkaline soil, alkaline effluent, alkaline sludge and oil industry wastewater samples in Jhagadia. (Jain et al., 2013) another study is done on the potential of an alkaliphilic bacterium *Klebsiella* sp. strain RJ-03 which is isolated from a soil sample of oil contaminated site from Gondal, to utilize different carbon sources for the production of an extracellular biosurfactant.

Donio et al., (2013b) reported the isolation of halophilic *Bacillus* sp. BS3 from solar salt works. He found that the produced biosurfactant has antiviral activity against shrimp white spot syndrome virus (WSSV) and anticancer activity performed in the mammary epithelial carcinoma cell at different concentrations of biosurfactants, the study revealed that 0.25 microgram concentration suppressed the cells significantly ($P<0.05$) to 24.8%. In other study Donio et al., (2013a) found that the halophilic bacteria *Halomonas sp* BS4 isolated from Thamaraikulam solar salt works in India, produce biosurfactant which is effectively controlled the human pathogenic bacteria and fungi an aquaculturally important virus, WSSV. The biosurfactant also repressed the proliferation of mammary epithelial carcinoma cell by 46.77% at 2.5 μg concentration.

Sarafin et al., (2014) found that Halophilic bacterial genera isolated from Kovalam solar salt works in Kanyakumari of India are screened for biosurfactant production, among eight of them *Kocuria sp*. (Km), *Kurthia sp*. (Ku) and *Halococcus sp*. (Hc) were found to have positive biosurfactant activity.

Mehetre et al., (2019) reported that Thermophilic and thermo-tolerant bacteria isolated fromUnkeshwar hot spring (India), four potent strains obtained which were identified as *Aeribacillus pallidus* (UCPS2), *Bacillus axarquiensis* (UCPD1), *Bacillus siamensis* (GHP76) and *Bacillus subtilis* subsp. *inaquosorum* (U277) and All the four strains were found to produce biosurfactants.

Plaza et al., (2006) reported the isolation of sixteen bacterial strains from petroleum hydrocarbons contaminated soils. All isolates reduced surface tension at varying degrees with strains, the surface tension reduction was a good measure of biosurfactant production.

Gugliandolo et al., (2003) another study revealed to the isolation of thermophilic, halophilic, aerobic spore-forming strain 4-1T, isolated from the water of a shallow sea hot spring at Vulcano Island (Italy), the strain showed a property of a surfactant activity.

Elazzazy et al. (2015) were isolated *Virgibacillus salarius* from soil and sea water samples, which were collected from Jeddah region, Saudi Arabia, *Virgibacillus salarius* showed ability to produce biosurfactant at wide range of pH, salinities and temperatures.

Gomes et al., (2016) study aimed to identify and characterize 141 halophilic bacteria isolated from production water and activated sludge from Marine Terminal Almirante Barroso (Brazil). Eleven strains showed significant ability for reduction of the surface tension.

Brakat et al., (2017) reported isolation of biosurfactant producers *Bacillus amyloliquefaciens* SH20 and *Bacillus thuringiensis* SH24 from Twenty-one oil-spilled seawater samples were collected from Shalateen, Red Sea, Egypt.

Recently Yaraguppi et al., (2020) reported the production of biosurfactant from *Bacillus aryabhattai* strain ZDY2 and they found that the biosurfactant showed antimicrobial activity against *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* and *Candida tropicalis*.

Objectives

1. Isolation of halophilic bacteria from marine water and soil samples near to marine ecosystem.
2. Screening of isolated halophilic bacteria for biosurfactant production potential.
3. Evaluation of biosurfactant producing halophilic bacteria for antibacterial, antifungal and antibiofilm activity.

4. Identification of promising isolate at molecular level.
5. Production, optimization and purification of biosurfactant.
6. Characterization of biosurfactant.
7. Study the bioactivities of biosurfactant (antibacterial, antifungal and antibiofilm).

Methodology to be adopt:

1. The water and soil samples for isolation of halophilic bacteria will be collected from marine ecosystems. The sample collection was carried out by composite random sampling in sterile polythene bags or botteles.
2. Isolation will be carried out onto sterile nutrient agar containing 5 to 20% NaCl or Zobell marine agar (Himedia). Screening of biosurfactant production from isolated halophilic bacteria will be carried out by drop collapse Test, surface tension measurements, Oil spreading technique, Emulsification activity by adding kerosene and equal volume of cell free supernatant and Hemolytic activity in 5% blood agar plate according to (Donio et al., 2013a; Sarafin et al., 2014).
3. Screening of potential biosurfactant producing halophilic bacteria for antibiofilm, antibacterial and antifungal activity will be carried out by microtiter plate method and agar well diffusion assay respectively. The test organisms including bacteria and fungi will be collected from standard culture providing institutes like, IMTech or MTCC (Giri et al., 2019; EK & Jyothis., 2020).
4. Identification of promising isolate at molecular level. Genomic DNA (100 ng) isolated from halophilic bacteria was amplified by PCR using 16 S rRNA universal primers.
5. By using a specified medium production of biosurfactant will be carried out according to (Brakat et al., 2017), and purification of produced biosurfactant will be done by using

analytical methods according to (Sarafin et al., 2014). The production conditions were optimized using OFAT and statistical approach.

6. For the characterization of produced and partially purified biosurfactant techniques like TLC, FTIR, GC–MS analysis, etc. will be performed.
7. The bioactivities of biosurfactant (antibacterial, antifungal and antibiofilm) will be studied in detail against selected pathogens to determine MIC and analytical tools like SEM will be used to assess its antibiofilm activities.

Importance of study or Society application:

International status

Giri et al., (2019) reported the antibacterial and anti-biofilm potential of biosurfactants isolated from *Bacillus subtilis* VSG4 and *Bacillus licheniformis* VS16. (Yan et al., 2019) Biosurfactants produced by *Pediococcus acidilactici* and *Lactobacillus plantarum* inhibited adhesion and biofilm formation of *Staphylococcus aureus* in a dose-dependent manner and affect expressions of biofilm-related genes (cidA, icaA, dltB, agrA, sortaseA and sarA) and interfere with the release of signaling molecules (AI-2) in quorum sensing systems. (Ceresa et al., 2015) has recently reported biosurfactant from *Lactobacillus brevis* CV8LAC which also has antibiofilm activity upto 90%, and has shown antifungal activity upto 60% when used for pre-coating of silicone elastometric disks.

National status

Yaraguppi et al., (2020) found that the biosurfactant from *Bacillus aryabhattai* strain ZDY2 showed antimicrobial activity against *Salmonella typhimurium, Escherichia coli, Staphylococcus aureus, Micrococcus luteus* and *Candida tropicalis*. (Pradhan et al., 2014) reported a new

glycolipid obtained from *Lysinibacillus fusiformis* S9 with potential anti-biofilm activity against pathogenic strains *E.coli* and *S. mutans* with no effects on microbial cell viability.

Donio et al., (2013b) report that the biosurfactant from halophilic *Bacillus* sp. BS3 has antibacterial activity which was16.00, 14.06, 13.10 and 10.10 mm of zone of inhibition against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi,* respectively. Also higher antifungal activity observed against *Trichophyton rubrum, Aspergillus niger* less activity against *Aspergillus flavus* and *Fusarium sp*. respectively

Significance of study

The proposed research work deals with isolation of halophilic bacteria, which have potential to produce biosurfactant with biological activity as antibacterial, antifungal and antibiofilm. The biosurfactants open new dimension to produce biomolecules have activity against pathogenic fungi, drug resistant bacteria and the formation of biofilm of pathogenic bacteria.

Year-wise Plan of work and targets to be achieved

Year	Target to be achieved
First Year	✓ Reference collection. ✓ Sample collection. ✓ Isolation of halophilic bacteria. ✓ Screening of isolates for biosurfactant production potential.
Second Year	✓ Screening of selected isolates showing antibacterial, antifungal and antibiofilm activities. ✓ Identification of promising isolate at molecular level. ✓ Optimization and purification of biosurfactant. ✓ Characterization of produced biosurfactant.
Third Year	✓ Study the bioactivities of biosurfactant (antibacterial, antifungal and antibiofilm) in detail. ✓ Project completion report. ✓ Publication, Communication

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